Mammary Excretion of 20-Methylcholanthrene*

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Recently, evidence was reported for the belief that a carcinogenic agent, 20-methylcholanthrene, could be secreted into the milk of a lactating animal and thereby gain entrance into the bodies of the offspring to cause an abnormally high incidence of neoplasms (1). Further positive evidence for this mode of excretion of methylcholanthrene has now been obtained by means of ultraviolet absorption spectroscopy, a method which has been used widely to follow the distribution of this and other carcinogens in animal tissues (2).

In the detection of methylcholanthrene in tissues of rats, advantage was taken of its high absorption peaks at 284 and 295 mµ. A solution of 1 µg. per milliliter in petroleum ether has an optical density (log 100/per cent transmission) of .250 at 284 and .312 at 295 mµ (measurements made with a Beckmann model DU spectrometer). The sensitivity of this method is limited by the fact that the unsaponifiable matter of rat tissues displays rather high general absorption in the ultraviolet, which increases with decreasing wave length (Fig. 1). As shown in Figure 1, however, the addition of methylcholanthrene in a concentration of 0.1 µg. per milliliter can be readily detected by inflections in the absorption curve of such an extract. Since the absorption of solutions of the unsaponifiable matter of a whole rat is such that it can be conveniently measured in a dilution of about 100 ml., the presence of methylcholanthrene in the bodies of rats in the amount of 10 µg. should be detectable by inflections at 284 and 295 mµ; and amounts of approximately 50 µg. should appear as discernible peaks on the absorption curve. In the present work, inflections at 284 and 295 mµ were taken as qualitative evidence for the presence of methylcholanthrene, with the recognition, of course, of the possibility that part or all of the absorption at these wave lengths may be due to metabolic products of methylcholanthrene.

METHODS AND RESULTS

Oral administration.—A female rat 24 hours post partum received 8 mg. of methylcholanthrene by stomach catheter in 1 ml. of olive oil, and this dose was repeated daily for 10 days. During this period a good deal of care was taken to prevent contamination of the suckling rats with the maternal feces. The possibility of the ingestion of maternal feces by rats 10 days of age seemed rather re-

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mote, since at this age they show no interest in food other than maternal milk. To minimize such a possibility, however, the animals were kept on wide-mesh screen, and any feces which remained were removed on daily inspection of the cages. To avoid including any of the carcinogen which may have contaminated the skin of the young rats through possible contact with maternal feces, special care was taken, during skinning, to avoid contact of the skinned carcass with the fur. Twenty-four hours after the last administration, when the methylcholanthrene added to a rat carcass Dilution of the alkaline solution with water or repeated extraction of the solution with petroleum ether increased background absorption and is unnecessary, since it was found that a single extraction with petroleum ether removes more than 90 per cent of the methylcholanthrene from a solution in 90 per cent ethanol.

The young were skinned, and gastro-intestinal tracts and skinned carcasses were separately pooled, extracted with alcohol, saponified, and extracted with petroleum ether as described above. The curves in Figure 2 indicate that methylcholanthrene can indeed be excreted in the milk. The presence of inflections in the absorption curves at 284 and 295 m\(\mu\) of the extracts of the carcasses and intestinal tracts of the young is taken as definite evidence for the presence therein of the carcinogen. By comparing this curve with those in Figure 1, we can make a rough estimation of the quantity of the carcinogen present, the total amount in carcasses and intestinal tracts of the whole litter being about 50 \(\mu\)g.

**Intravenous and intraperitoneal administration.** —The absorption curve for the mother's carcass (Fig. 2) showed that only extremely small quantities of methylcholanthrene were being absorbed from the intestine, a finding which is in good agreement with the recent observations of Heidelberger and Jones (3) with radioactive 1,2,5,6-dibenzanthracene. Since transfer of the carcinogen to the offspring by way of the milk was doubtless being limited by its absorption from the gastro-intestinal tract of the mother, it was anticipated that intravenous or intraperitoneal administration of the carcinogen would raise the levels considerably and hence would increase its mammary excretion. This expectation was borne out as shown by the absorption curves in Figure 3. For intravenous administration, an emulsion of the carcinogen was injected into the tail vein. The emulsion was prepared as follows: a solution of 20 mg. of methylcholanthrene in about 3 ml. of acetone was added in small portions, with stirring, to a boiling solution of 50 mg. Emulsone B (a nontoxic emulsifying agent manufactured by the Glyco Products Co.) in 10 ml. of water. The emulsion was then concentrated to 10 ml., yielding a fairly stable nontoxic suspension with a final concentration of 2 mg. methylcholanthrene per milliliter.

A female rat, nursing a litter of seven, was given 1 ml. of the emulsion intravenously on the tenth day post partum. Five hours later the mother and litter were sacrificed and separately extracted as described previously. The inflections at 284 and 295 m\(\mu\) on the absorption curves indicated the

**FIG. 2.**—Absorption curve of extract of unsaponifiable matter of bodies of young whose mother received methylcholanthrene by oral instillation. Curve A, extract of young; curve B, extract of mother.

young were 12 days old, the mother and her litter of ten were sacrificed. The mother was carefully skinned and the entire gastro-intestinal tract removed. The carcass was ground into 95 per cent alcohol and extracted continuously with alcohol for 8 hours. The extract was saponified by the addition of sufficient potassium hydroxide to give a final 1-M solution followed by refluxing 2 hours. After cooling, the alkaline alcoholic solution, without the addition of water, was extracted directly with a single portion of petroleum ether. It was found that this procedure quantitatively recovered
presence of the carcinogen in the bodies of the young rats; the amount was roughly estimated at 40 μg. In similar experiments after intraperitoneal injection of 8 mg. of the carcinogen in 1 ml. of olive oil, it was again demonstrable in the bodies of the young; the amounts present ranged from about 30 μg., in those sacrificed 3 hours after injection of the mother, to approximately 230 μg. in those sacrificed 24 hours after the injection.

In two experiments, one after intravenous, the other after intraperitoneal injection, evidence was obtained for the presence of the carcinogen in the milk. In each experiment, 5 hours after injection of 2 mg. of methylcholanthrene in the mother, the young were sacrificed and the milk removed as a coagulated mass from the stomachs. After extraction and saponification essentially as described for the other tissues, extracts of the unsaponifiable matter of the milk gave the curves shown in Figure 4. The amounts of methylcholanthrene present therein are estimated to be of the order of 20–30 μg.

To test the possibility that the excreted material was a degradation product rather than the intact hydrocarbon, an experiment was performed with radioactive methylcholanthrene. By isolating pure radioactive methylcholanthrene from the milk by the carrier technic, it was possible to establish definitely that this carcinogen can be transferred intact by way of the milk to the offspring. The radioactive 20-methylcholanthrene, labeled with C¹⁴ in the 11-position, was obtained through the kindness of Dr. W. G. Dauben of the Department of Chemistry of the University of California. It had an activity of 100,000 counts/min/mg, which in our Q gas counter corresponded to a specific activity of 280,000 counts/min/dish.

A rat, 8 days post partum, received 5 mg. of the radioactive carcinogen by intraperitoneal injection in 2 ml. of olive oil. After 5 hours, during which time the young nursed freely, the entire litter of eight was sacrificed. Each one was carefully skinned, the stomachs opened, the milk contained therein pooled, and the unsaponifiable matter extracted as described above. This material, in an amount of 28 mg., gave an absorption curve similar to Figure 4, corresponding to approximately 5 μg. of hydrocarbon. When counted on a 7.5 sq.
cm. dish in a flow counter, it had an activity of 39 counts per minute above background. To this material there was added 10 mg. of nonradioactive carrier methylcholanthrene, and the mixture crystallized from alcohol. The specific activity, corrected for layer thickness, was 63 counts/min/dish. On a second crystallization from alcohol, the activity increased to 71 counts/min; and on the third crystallization from acetone, the activity remained essentially constant at 68 counts/min. By a similar procedure it was also possible to demonstrate the presence of the intact carcinogen in the skinned carcasses of the young. On the basis of the specific activity of the original methylcholanthrene of 280,000 counts/min/dish, we can calculate that the milk contained $68 \times 10,000/280,000 = 2.5 \mu g$ of the carcinogen. This value is similar in magnitude to the value of $5 \mu g$ obtained from ultraviolet absorption. This, of course, represents only a minute fraction of the total quantity of 5 mg. administered, but the data evidently leave no further doubt concerning the mammary excretion of methylcholanthrene.

The mammary excretion of a polycyclic hydrocarbon such as methylcholanthrene is not surprising, in view of the generally well recognized excretion of other lipoids by this route. The fact has long been known that when pungent foodstuffs are eaten by a lactating mother, the essential oils which carry the flavors find their way into the milk. The mammary excretion of vitamin D (4), estrogens (5, 6), and a host of drugs such as DDT (7), thiourea and thiouracil (8), and sulfathiazole (9) have been reported, and there is no reason why such lipid-soluble substances as the carcinogenic hydrocarbons should not behave similarly.

The carcinogenic activity of methylcholanthrene when administered in this fashion by way of the mother's milk is now under further investigation.

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